



mPEMMR (SA) User Guide



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I. System Requirement

- RAM 4GB or More
- JDK 1.5 or More (64bit Recommended)
- TPP (<http://tools.proteomecenter.org/wiki/index.php?title=Software:TPP>)
- msconvert
- Thermo Foundation 2.0 (for Q-Exactive)
- Xcalibrabur 2.2 (for Q-Exactive)

II. Installation

1) Unzip mPEMMR_1.1.8.zip

- log4j (directory)
- LIB (directory)
- RAPID1.07 (directory)
- mPEMMR_1.1.8.cmd (Command Executable File)
- mPEMMR_param.txt (mPEMMR Parameter File)
- UserGuide.pptx (User Guide)

2) mPEMMR_1.1.8.cmd : Open file in text editor to modify as below

```
@echo off
```

```
SET PEMMR_HOME=D:\Programs\mPEMMR_1.1.8 (mPEMMR.cmd Change to file existing Directory Address)
```

```
SET CLASSPATH=%PEMMR_HOME%\LIB\mPEMMR_1.1.8.jar;%PEMMR_HOME%\msfile_3rd_party\jnzreader-1.2.0.jar;%PEMMR_HOME%\msfile_3rd_party\mxml-parser-1.3.2.jar;%PEMMR_HOME%\msfile_3rd_party\xxindex-0.11.jar;%PEMMR_HOME%\msfile_3rd_party\com.springsource.org.apache.commons.logging-1.1.1.jar;%PEMMR_HOME%\msfile_3rd_party\com.springsource.org.apache.commons.io-1.4.0.jar;%PEMMR_HOME%\msfile_3rd_party\log4j-1.2.13.jar;%PEMMR_HOME%\msfile_3rd_party\jcommon-1.0.16.jar;%PEMMR_HOME%\log4j\log4j-1.2.15.jar;
```

```
set MEM=8G
```

```
if ""%1""=="*" goto example
```

```
goto exec1
```

```
:example
```

```
echo Example) mPEMMR_1.1.8 [mPEMMR_Params.txt]
```

```
goto end
```

```
:exec1
```

```
java -Xmx%MEM% pemmr_sa.PEMMRExec %*
```

```
goto end
```

```
:end
```

III. Program Execution

- 1) Modify *mPEMMR_param.txt* as below
- 2) Run Command line
- 3) Change directory file of *PEMMR.cmd* (In this case, D:\WPrograms\WPEMMR_SA_Release\W1.1)
- 4) Run the program typing *PEMMR.cmd pemmr_sa_param.txt*
- 5) PEMMR will create *xxxx_PEMMR.mgf* which will be used for peptide search

mPEMMR_param.txt

```
##### External Programs #####  
RAPID=D:/Programs/mPEMMR_1.1.8/RAPID1.07/RAPID.exe  
MSCONVERT=C:/Inetpub/tpp-bin/msconvert.exe  
MZXML2SEARCH=C:/Inetpub/tpp-bin/MzXML2Search.exe  
##### UMC Create #####  
RAW_FILE_URL= D:/Programs/mPEMMR_1.1.8 /Example/N1_5ug_100cm_300min_1_091112.raw  
UMC_ALGORITHM=1  
SCAN_COUNT=2  
HOLE_COUNT=5  
MASS_TOLERANCE=10  
MIN_MASS=0  
MIN_INTENSITY=0  
##### Spectrum Filtering #####  
SCAN_RANGE=5  
UMC_LINK_TOLERANCE=10  
### since ver 1.1.7 (ppm) ###  
ISOTOPE_ENVELOPE_TOLERANCE=5
```

"Multiplexed Post-Experimental Monoisotopic Mass Refinement (*mPE-MMR*) to increase sensitivity and accuracy in peptide identifications from tandem mass spectra of co-fragmentation" Madar, I. H.; Ko, S.-I.; Kim, H.; Mun, D.-G.; Kim, S.; Smith, R.; Lee, S.-W. *Anal. Chem.* 2017, 89(2), 1244-1253.

III. Program Execution (Editing pemmr_sa_param.txt)

mPEMMR_param.txt

```
##### External Programs #####  
RAPID=D:/Programs/mPEMMR_1.1.8/RAPID1.07/RAPID.exe  
MSCONVERT=C:/inetpub/tpp-bin/msconvert.exe  
MZXML2SEARCH=C:/inetpub/tpp-bin/MzXML2Search.exe  
##### UMC Create #####  
RAW_FILE_URL= D:/Programs/mPEMMR_1.1.8 /Example/N1_5ug_100cm_300min_1_091112.raw  
UMC_ALGORITHM=1  
SCAN_COUNT=2  
HOLE_COUNT=5  
MASS_TOLERANCE=10  
MIN_MASS=0  
MIN_INTENSITY=0  
##### Spectrum Filtering #####  
SCAN_RANGE=5  
UMC_LINK_TOLERANCE=10  
### since ver 1.1.7 (ppm) ###  
ISOTOPE_ENVELOPE_TOLERANCE=5
```

- RAPID program is provided with mPEMMR and it is located in PEMMR_HOME sub directory
- MSCONVERT and MZXML2SEARCH is installed with TPP
- RAW_FILE_URL is URL of RAW file under execution

Caution : All directories typed in mPEMMR_param.txt file should be entered using "/"

e.g.)RAPID=D:\Programs\PEMMR_SA_Release\1.1\RAPID1.07\RAPID.exe (X)
RAPID=D:/Programs/PEMMR_SA_Release/1.1/RAPID1.07/RAPID.exe (O)

IV. Troubleshooting

Q. Stop running with the message of "*java.lang.ClassNotFoundException*"

A. Open PEMMR.cmd file in text editor and make sure the dir of "SET PEMMR_HOME location" entered properly.

Q. Execution is interrupted with the message "*Exception in thread "main": java.lang.OutOfMemoryError: Java heap space*"

A. Open PEMMR.cmd in text editor and increase MEM to 8G.

Q. RAPID doesn't run properly and the file size of resultant files (_isos.csv, _scans.csv) is 0.

A. RAW file has not read properly by the program, Re-Install the Thermo foundation.

If mPEMMR program stopped generating result files, need to delete the existing generated files and run the PEMMR once again